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Valverde Pérez, Borja; Xing, W.; Zachariae, A. Z.; Kjeldgaard, A.F. ; Skadborg, M.M.; Palomo, Alejandro; Smets, Barth F.

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Microbial protein production using a novel bubble-free membrane bioreactor

Valverde-Pérez, B.*, Xing, W., Zachariae, A.Z.*, Kjeldgaard, A.F., Skadborg, M.M.*, Palomo, A.*, and Smets, B.F.*[1]**
* Technical University of Denmark, Department of Environmental Engineering, Building 113, DK-2800 Kgs. Lyngby, Denmark (Corresponding author: bfsm@env.dtu.dk)
** School of Civil Engineering, Beijing Jiaotong University, Beijing 100044, China

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Summary of key findings

This work demonstrates the applicability of a novel bubble-free membrane bioreactor for cultivation of methanotrophic bacteria for single cell protein production. The methane and oxygen supply were optimized, so they were in contact only in the liquid phase thereby avoiding the creation of explosive atmospheres. After optimization of gas supply, the biomass accumulated protein up to 51 % of its dry weight. The microbial protein contained most of the essential amino acids needed to serve as animal feed.

Background and relevance

Global population increase, climate change and industrialization are leading to alarming depletion rates of many resources worldwide. Consequently, there is an increasing interest in recovering resources from waste streams, which have traditionally not been utilized or negatively valued (Foley et al., 2011). Conventional strategies for valorisation of bio-waste focus mainly on the production of biogas and recovery of nutrients as, e.g., compost or slurries that can be used as fertilizers. However, fertilizer application on land is not efficient because it comes with large nutrient losses (about 50% of the total nitrogen input) as greenhouse gas emissions or run-off, potentially leading to additional pollution. Most agronomic crops are used as protein source for livestock for human consumption. Therefore, the direct production of feed-grade proteins from residual streams, rather than the production of fertilizers to support cultivation of protein-rich agronomic crops, would be desirable (Matassa et al., 2015).

Single-cell protein (SCP) consisting of microbial biomass can provide nutritive proteins, with quality equal or better than conventional protein sources (e.g. soy or fishmeal) and at a lower cost. Some attempts have been made to produce SCP from synthetic or fossil fuel based resources (referred to as first generation processes). As example, methane oxidizing bacteria (MOB) are used at large scale for SCP production using natural gas and synthetic nitrogen sources. MOB can be grown in effluents from anaerobic digestion, enabling nutrient and carbon recovery into valuable feed ingredients. However, there are still limitations related with gas supply, which is usually inefficient and creates explosive mixtures of methane and oxygen (Petersen et al., 2017).

In this study we developed a novel bubble free membrane-based bioreactor (Fig.1) for safe and efficient MOB cultivation to produce SCP. The system was started-up as a batch reactor and then operated as a continuous flow system. Methane and air were supplied separately to the reactor using highly-efficient hydrophobic hollow-fibre membrane modules (Mitsubishi Rayon Co., Ltd., Tokyo, Japan). In this way methane and oxygen were only in contact solubilized in the liquid phase, avoiding the formation of explosive atmosphere inside the reactor. The inoculum consisted of a mixed methanotrophic culture previously used by Van der Ha et al. (2011) and was grown on a synthetic medium. The reactor was operated for 2 periods of 100 and 90 days, respectively. Dissolved oxygen, optical density (600 nm), dissolved methane, ammonia, nitrite, nitrate, pH and temperature were monitored. Microbial composition was characterized via 16S rRNA gene amplicon sequencing. Gas emissions from the reactor and their composition were also monitored to detect gas leakages.

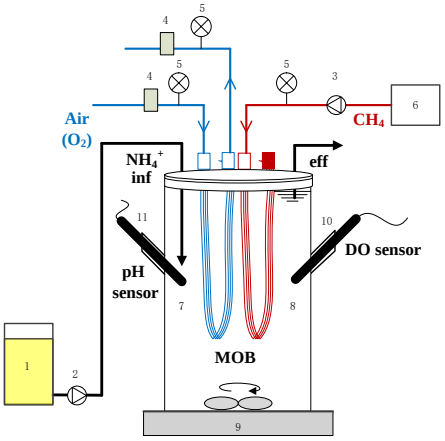

Figure 1. Schematic representation of the bubble-free membrane-based reactor used for cultivation of the mixed MOB culture.

Results

During the first operational period, the oxygen level in the reactor was relatively low (approx. 0.1 mg L^{-1}), which led to preferential biofilm growth on the membranes. The culture was characterized (Fig. 2)and *Methylobacter* was the main MOB , followed by *Methylomonas*. *Methylophilus*, a methylotroph (able to use both methane and methanol), was also abundant during the first operational period. Interestingly, both could grow in the system even when methane supply was stopped. MOB probably oxidized ammonia to nitrite in the absence of methane. The mixed culture had good protein quality, which could serve as an alternative protein source for, e.g., fish (Fig. 3; Yamamoto et al., 2005). Both methanotrophic biomass and microbial protein content decreased during operation, suggesting that protein content related mostly to MOB. The final protein content was 15.4% of total biomass, which is unsuitable for substitution of traditional protein sources (Yamamoto et al., 2005; Schøyen et al., 2007).

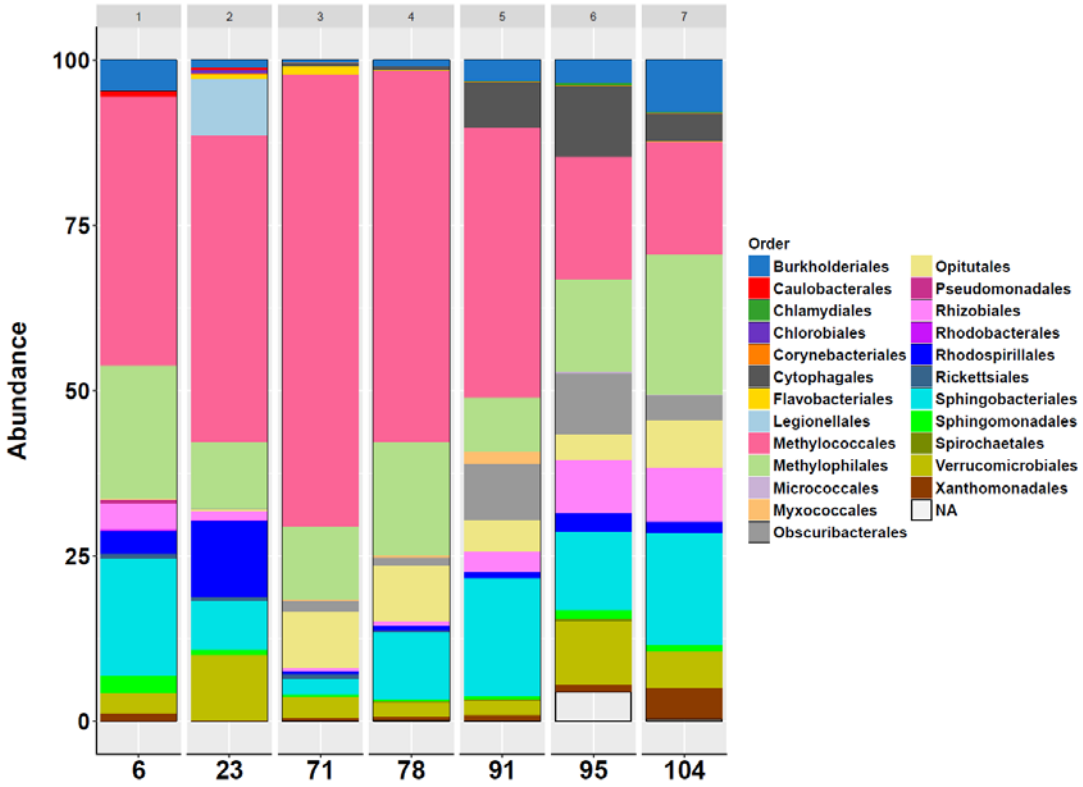

Figure 2. Order-level taxonomic classification of 16S rRNA amplicons at selected days of the reactor operation. Taxa abundance is expressed in percentage.

During the second period, the oxygen supply to the system was improved by doubling the surface area of the membranes supplying oxygen. Increasing the oxygen level above 0.5 mg L^{-1} supported microbial growth in suspension and fouling was minimized. After stable operation was achieved, oxygen was supplied as pure oxygen with a dead end membrane system. In parallel, the methane supply was doubled with respect to the initial operational conditions tested in periods 1 and 2. Oxygen levels were relatively higher, but were challenging to control manually. Despite the fact that we could not manually keep the reactor stable, it should be noticed that simple control strategies, e.g. on-off aeration control, could be easily implemented to keep oxygen stable within a reasonable range (e.g., 0.5 to 3 mg L^{-1}). Then main benefit of these operational conditions is the minimum leakage of gases to the reactor. While the reactor was supplied with pure oxygen, the gas leakage was 170 ml day^{-1}, which is about 4 times lower compared to the periods when atmospheric air was used as oxygen source. Nevertheless, in both scenarios the methane leakages was lower than 0.004% of the total methane supplied. Most of the emitted gases were both nitrogen (unused gas fraction from air) and carbon dioxide (emitted as product from methane oxidation). The protein content increased in the last period up to 51% of the biomass, containing most of the essential amino acids to feed, e.g., fish or chicken (Fig. 3, Schøyen et al., 2007; Øverland et al., 2006). We are currently analysing the sequences of the second period to further support our observed correlation between methanotrophic biomass and protein content.

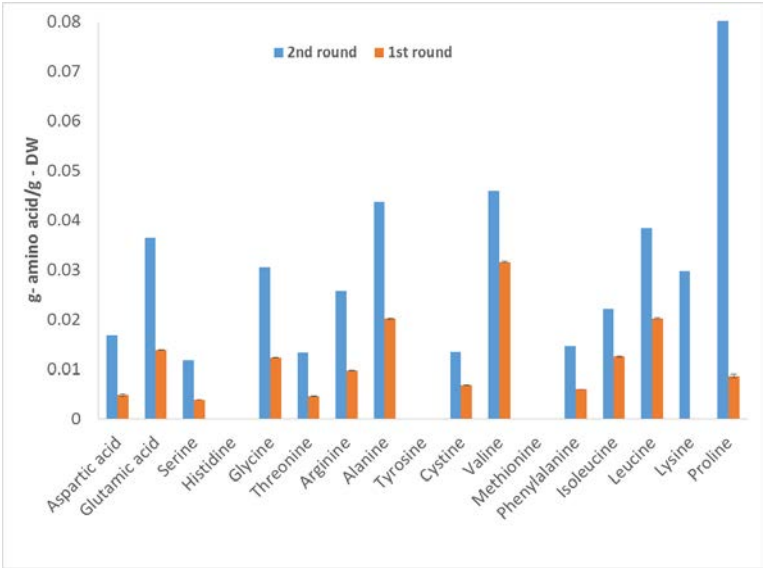

Figure 3. Amino acid profile of the methanotrophic enrichment culture grown in the bubble-free bioreactor.

References

Foley, J.A., Ramankutty, N., Brauman, K.A., et al., 2011. Solutions for a cultivated planet. *Nature*, 478.

Billing, A.E. and Dold, P.L. (1988a) Modelling techniques for biological reaction systems, *Water SA*, 14(4), 185–192

Matassa, S., Batstone, D.J., Hülsen, T., Schnoor, J., Verstraete, W., 2015. Can direct conversion of used nitrogen to new feed and protein help feed the world? *Environ. Sci. Technol.* 49, 5247–5254.

Petersen, L. A. H., Villadsen, J., Jørgensen, S. B., Gernaey, K. V, 2017. Mixing and mass transfer in a pilot scale U-loop bioreactor. *Biotechnol. Bioeng.* 114, 344–354.

Schøyen, H., Svihus, B., Storebakken, T., Skrede, A., 2007. Bacterial protein meal produced on natural gas replacing soybean meal or fish meal in broiler chicken diets. *Archives of Animal Nutrition*, 61(4), 276-291.

Van der Ha, D., Bundervoet, B., Verstraete, W., Boon, N., 2011. A sustainable, carbon neutral methane oxidation by a partnership of methane oxidizing communities and microalgae. *Water Research*, 45(9), 2845–2854.

Yamamoto, T., Sugita, T., Furuita, H., 2005. Essential amino acid supplementation to fish meal-based diets with low protein to energy ratios improves the protein utilization in juvenile rainbow trout Oncorhynchus mykiss. *Aquaculture* 246, 379–391.

Øverland, M., Romarheim, O.H., Hovin, M., Storebakken, T., Skrede, A., 2006. Apparent total tract digestibility of unprocessed and extruded diets containing basic and autolyzed bacterial protein meal grown on natural gas in mink and rainbow trout, *Anim. Feed Sci. Technol.* 129 237–251.

Presenting Author

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Barth F. Smets
Department of Environmental Engineering, Technical University of Denmark

Is the presenting author an IWA Young Water Professional? N

Bio:

Barth F. Smets is Professor of Environmental Microbiology at the Technical University of Denmark. His research focuses on Microbial Resource Management and Engineering: the bridge between environmental engineering and microbial ecology. His research group uses both advanced experimental (microscopic, molecular, omic) tools as well as computational (agent and continuum models) approaches to study fundamental and applied microbial ecological questions, with a focus on mixed microbial communities within water engineering applications. Central interests are the ecology of antibiotic resistance genes, the microbial ecology of drinking water production systems, dynamics and control of nitrogen-fueled microbial communities, and new biotechnologies for nutrient recovery, and a general interest in the forces that shape and control microbial communities and their activities. He has ca. 180 ISI publications, with an H-index of 36.